Faecal urobilinogen levels and pH of stools in population groups with different incidence of cancer of the colon, and their possible role in its aetiology[1]

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Summary: Mean faecal urobilinogen levels and the pH of stools were both found to be higher in subjects from a population group at high risk of developing cancer of the colon than in subjects matched for age, sex and socioeconomic status from a low-risk population group. An alkaline reaction of the colon contents seems to have a tumorigenic effect by a direct action on the mucus of the mucous cells. An acidic reaction, on the other hand, appears to be protective. These differences are dependent on the patterns of diet and manner of eating. Proper mastication of food, roughage, cellulose and vegetable fibre, and short-chain fatty acids of milk and fermented milk products in the diet appear to be protective.

Introduction

It has been suggested that bile and its derivatives play an important role in the aetiology of cancer of the large intestine. Two possible factors in the aetiology have been investigated. One is that bile acids are converted by the gut bacteria into chemical carcinogens which produce tumours by a direct contact action (Reddy & Wynder 1977, Hill 1975, Reddy *et al.* 1980). The other is that bile and its derivatives cause colonic cancer by a change in the pH of the colonic contents brought about by variations in the amount and flow rate of bile and pancreatic juice entering the intestinal lumen (Malhotra 1967*a*, 1977*a*, 1981).

The incidence of cancer of the colon is higher in South Indians than in North Indians in the Punjab, with the exception of Agra in the northern state of Uttar Pradesh (Paymaster 1964, Malhotra 1967*a*). This paper reports the faecal urobilinogen levels and the pH of stools in subjects from both these high and low-risk groups. The possible role of an alkaline milieu surrounding the mucous cells of the colon in the development of cancer of the colon has been suggested previously (Malhotra 1967*a*, 1976, 1977*a*, 1981, 1982 in preparation).

Methods

High-risk group: Sixty South Indian male railway sweepers, aged between 40 and 58 years (average 51 years), who worked at the Perambour Integral Coach Factory at Madras in South India and the railway colony at Madras on the Southern Railway, were randomly selected as the high-risk group. Their habitual diet consisted of non-masticatory meals of boiled rice, ragi gruel (*Eleusine coracoma*), tapioca and tamarind water, low in fibre and low in milk and fermented milk products such as yoghurt and ghee.

Low-risk group: Sixty randomly-selected North Indian adult males from the Punjabi Sikh community, aged between 40 and 58 years (average 53 years), working as carpenters in the Wagon Repair Shops at Kharagpur on the South Eastern Railway, comprised the low-risk group. Their habitual masticatory diet consisted of high-fibre wheat staple, chick peas, legumes (*Phaseolus mungo*), Raj Maanh (*Vigna sinensis*), whole beans and other legumes (dahls), with milk and fermented milk products such as yoghurt, ghee and white cheese.

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Laboratory investigations: Faecal urobilinogen excretion (mg/24 hours) was estimated in duplicate by the method of Maclagan (1946) as suggested by Varley (1963), and according to the protocols described previously (Malhotra 1967*b*, 1968*a*,*b*).

The pH of stools was determined on a homogenized specimen of stool using a combination of wide and narrow range Universal paper strips for pH. The readings obtained were comparable with those obtained with the Beckman pH meter using glass electrodes in 30 random samples of unformed, semi-liquid stool specimens.

Results

The mean values for both faecal urobilinogen excretion and the pH of stools were found to be higher in the South Indian railway sweepers (high-risk group) than in the North Indian Punjabi railway carpenters (low-risk group), as can be seen from Table 1. Comparison between the two groups according to the distribution of pH values showed that in the high-risk group there was a preponderance of stool samples in the higher pH ranges, whilst in the low-risk group most stool samples were in the lower pH ranges (Table 2).

Table 1. Faecal urobilinogen excretion and pH of stools in population groups at high risk (South Indian railway sweepers) and low risk (North Indian railway carpenters) of developing cancer of the colon

	South Indians (high risk) ($n=60$)	North Indians (low risk) ($n=60$)	Significance
Faecal urobilinogen excretion (mg/24 h; mean ± s.d.)	86.00 ± 14.21	61.99 ± 16.99	s.e. = 2.85, $t=8.4$, $P \leqslant 0.0001$
pH of stools (pH units; mean ± s.d.)	7.8 ± 1.14	6.5 ± 1.02	s.e. = 0.198, $t=6.56$, $P \leqslant 0.001$

Table 2. Distribution of stool samples at different pH ranges in high-risk (South Indian) and low-risk (North Indian) subjects

	pH ranges									
	⩽4.5	5.0 5.5	5.6 6.0	6.1 6.5	6.6 7.0	7.1 7.5	7.6 8.0	8.1 8.5	8.6 9.0	⩾9.1
High risk ($n=60$)	0	0	1	4	4	8	11	17	7	8
Low risk ($n=60$)	1	9	31	3	1	3	5	4	1	2

χ^2 test for independence, grouping intervals as indicated by braces, was carried out to obtain expected frequencies of not less than five: $\chi^2 = 60.03$, d.f. = 7, $P < 0.001$

Discussion

These disparate geographical groups were enlisted randomly as previous studies have shown a substantial difference in their tendency to develop cancer of the colon (Paymaster 1964); the disease is more than twice as frequent among South Indians than North Indians (Malhotra 1967*a*). It might be argued that railway workers are not representative of the general population and this is, therefore, a defect in this study. However, since we are interested in the comparison of certain characteristics of the high and low-risk groups, and not so much in absolute rates (which is the subject of a companion study: Malhotra 1982, in preparation), the investigation of South Indian railway sweepers and North Indian railway carpenters in this study is valid.

The use of mean faecal urobilinogen excretion for computing the amount of bile entering the intestinal lumen and its flow-rate is not entirely satisfactory, and the limitations of this have been discussed previously (Varley 1963, Hoffman 1970, Kernohan 1976, Malhotra 1967 *b*,*c*, 1968 *a*,*b*). Up to 50% of the urobilinogen of the colon is reabsorbed and returned to be re-excreted. Under normal circumstances the amount of urobilinogen excreted in the faeces depends upon the amount of bile entering the intestinal lumen (Varley 1963, Hoffman 1970).

Although the method used here does not give the absolute amount of bile entering the intestine, it is satisfactory for comparative purposes. One conclusion from the results is that significantly higher amounts of bile enter the intestines in the case of the high-risk South Indian sweepers compared with the low-risk North Indian carpenters.

Hepatic bile is alkaline (pH 7.8–8.6). On storage in the gallbladder it loses water as well as its bicarbonate content. Thus, the bicarbonate content of concentrated gallbladder bile is 5 mEq/1, whereas for bile flowing at the rate of 0.1 ml/min it is 10 mEq/1 (Magee 1965). It seems that among other factors, such as the alkaline pancreatic juice and the acidic H^+ ion liberated from the fermentation of cellulose and dietary fibre in the colon into acetic acid (Popjack *et al.* 1951) and other short-chain fatty acids (Glynsky *et al.* 1976, Cummings 1981), the higher pH of the stool samples from the South Indians is due to a larger amount of bile and pancreatic juice entering the intestinal lumen, and a lower H^+ ion concentration because of a lack of dietary fibre and short-chain fatty acids in the diet.

The method used to obtain the mean pH of stools, i.e. totalling the pH values and dividing the sum by the number of observations in each group (Mitchell *et al.* 1962), does not give the true mean pH. On the pH scale true mean pH can be derived only from the reciprocal of the anti-logarithm of the individual pH values, the value obtained being converted back to the pH scale as the 'mean pH'. However, since in this study we are comparing the pH in two different groups, obtained by the same method, the estimation of 'true mean acidity' is not essential. This method is not only convenient but is, in fact, preferable for statistical analyses, because it gives a more normal distribution about the mean (Lennard-Jones & Babouris 1965, Malhotra 1967*d*).

The distribution of pH values in the stool samples of the high-risk versus the low-risk group was then considered (Table 2). This clearly showed a preponderance of high pH stool samples in the high-risk group and low pH stool samples in the low-risk group. It is therefore reasonable to conclude that the faeces of the high-risk group were alkaline, whereas those of the low-risk group were slightly acidic.

The effect of an alkaline milieu on the intracellular mucus of the mucous cells has been studied by various workers with similar results. The mucus is rendered fluid and in that state it escapes from the cell (James 1957, Ball & James 1961, Malhotra 1967*e*). The relationship of the mucus to the mucous cells is unique in that it is adherent to the cell, forming an integral part of the cell, and is not just mucus smeared on the surface of the mucous membrane.

Lawson (1964) in experiments on dogs, and du Plessis (1965) in human studies, have shown that the loss of mucus from these cells results in hyperplasia, metaplasia, cell atypia and a striking 40-fold increase in mitotic activity. Although their observations were made on gastric mucosa, this also applies to mucous cells where the mucus is an integral part of the cell. For example, Dunham *et al.* (1966) demonstrated similar changes in the cheek pouches of hamsters by repeatedly painting them with a strong solution of calcium hydroxide. Similar changes occur in the uterine cervix due to an alkaline semen (Malhotra 1971); in the oral cavity due to the alkaline slaked lime in betel quid (Shanta & Krishnamurthi 1963, Hirayama 1966, Malhotra 1967*a*); and in the breast (Malhotra 1977*b*, 1982). Auerbach *et al.* (1974) noted similar changes in the lung parenchyma of cigarette smokers whose respiratory cells had metamorphosed from the normal ciliated to goblet cells due to chronic bronchitis; and cigarette smoke is alkaline in reaction (Malhotra 1970). Moreover, Dunham *et al.* (1974) induced similar chronic inflammatory changes, marked hyperplasia and carcinoma-in-situ in the oesophagus of hamsters treated with a quid containing a plant extract, arecoline and calcium hydroxide, and known to be carcinogenic. However, arceoline alone failed to produce any lesions. Some changes, viz., hyperplasia, cell atypia and marked increase in mitotic activity, are a prelude to neoplasia (Poel 1964).

Faecal urobilinogen levels are related to diet. Population groups such as the South Indians, who eat non-masticatory, low fibre foods, low in short-chain fatty acids of milk and fermented dairy products, have higher urobilinogen levels than the North Indians whose diets are masticatory wheat staple, high in fibre and high in fermented milk products such as yoghurt and ghee made by the fermentation process (Malhotra 1967*c*). Altering the diet alters

faecal urobilinogen excretion (Malhotra 1968*a*). In an attempt to isolate the effect of dietary constituents from the manner of eating (meal-chewing versus meal-scamping), volunteers given non-masticatory boiled rice were studied first following their habitual hurried pattern of eating, and on another occasion after eating by deliberately and thoroughly masticating the food; following the latter both faecal urobilinogen excretion and concentration were significantly reduced (Malhotra 1968*b*). The faecal weights were not altered. This does not accord with the view that fibre alone is responsible for such changes due to an increase in stool weight produced by it thus resulting in dilution of the bile acids.

Burkitt (1971) has suggested that fibre in the diet is associated with an increase in stool bulk and more rapid transit times, thus reducing the contact time of any chemical carcinogen. It has also been suggested that some forms of fibre will reduce the concentration of faecal bile acids by increasing their excretion. Eastwood *et al.* (1973) have contested both views; they showed that adding wheat bran and cellulose to the diet increased stool weight but did not alter the intestinal transit times significantly. Glober *et al.* (1977) have also disputed the 'transit time and dilution' hypothesis. They found that the Japanese living in Hawaii have bowel cancer rates similar to those of the indigenous American population but appreciably faster gut transit times, whilst the Japanese living in Japan have identical transit times with the Japanese living in Hawaii but a lower incidence of colonic cancer. Moreover, persons with severe constipation do not have an increased incidence of cancer of the colon.

Roughage, cellulose and vegetable fibre are important nonetheless, but their mode of action is entirely different. Fibre ferments in the gut liberating large quantities of acetic acid (Popjack *et al.* 1951) and other short-chain fatty acids such as propionic, lactic and butyric acids (Ling 1963, Glynsky *et al.* 1976, Cummings 1981). These volatile acids, especially acetic acid, act in two different ways. First, they affect the pH of the colon contents by virtue of their H^+ ion concentration. In an acidic milieu the mucus of the mucous cells is precipitated *in* the cell and it is thus prevented from escaping from the cell (James 1957). Secondly, acetic acid, being the precursor of all short-chain fatty acids (Ling 1963), increases the relative proportion of short-chain fatty acid glycerides in the plasma (Ahrens *et al.* 1958, Malhotra 1967*b*). This retards gallbladder contraction (Malhotra 1967*f*). Sarles (1965) showed that whilst the long-chain complex acids of C_{18-21} series caused a powerful contraction of the gallbladder, short-chain simple acids (C_4C_6) hardly produced any contraction. The effect of this in the case of meal-scampers is that by eating long-chain seed oils and other highly complex long-chain fats of beef and pork, the storage time of bile in the gallbladder is impaired and the flow rate of bile increases; this results in an increased bicarbonate content (Magee 1965) and hence increased pH. Gallbladder emptying rates are slower in meal-chewers eating meals rich in fibre and short-chain fatty acids compared with meal-scampers eating meals low in fibre and in short-chain fatty acids but high in long-chain fatty acid triglycerides (Malhotra 1967*f*).

Fibre acts in yet another way. It requires chewing and this increases the concentration of mucus in the saliva (Malhotra *et al.* 1965, Malhotra 1967*e*). Salivary mucus swallowed with meals is precipitated onto the gastric and duodenal mucosa in an almost impenetrable barrier when it comes into contact with the gastric HCl (James 1957). This prevents the secretogogues and the stimuli responsible for cholecystokinin-pancreozymin release from reaching the receptors. The overall effect is that in meal-chewers there is a decrease in the flow rate of bile and pancreatic juice which results in a decrease in alkalinity. This is further helped by acid inhibition of gastric juice by the salivary mucus (Malhotra 1972), HCl being the most potent duodenal stimulus for gallbladder contraction (Magee 1965). The physiological situation is just the reverse in the case of meal-scampers eating meals low in fibre and in short-chain fatty acid triglycerides but rich in long-chain ones. The overall effect is that cholecystokinin-pancreozymin causes a powerful contraction of the gallbladder resulting in the entry of alkaline bile and an alkaline pancreatic secretion into the intestinal lumen (Creamer 1978).

Besides these effects produced by dietary fibre, dietary short-chain fatty acids of dairy products in the diets of the North Indians counteract intestinal alkalinity and augment acidity. The diets of the South Indians, on the other hand, are lacking in these characteristics (Diet Atlas of India 1964, Malhotra 1967*b*).

Short-chain fatty acids have an antibacterial effect. Production of these acids in the colon is one of the mechanisms which prevents the establishment of pathogenic bacteria such as salmonella (Cummings 1981). Might this not suppress the colonic bacteria, which, it has been suggested, convert bile acids into chemical carcinogens? Thornton (1981) has put forward the view that acidification of colonic contents could be expected to reduce bile acid degradation by 7α-dehydroxylation. Could this be due to the suppression of colonic bacteria by short-chain fatty acids? If so, this is in keeping with the finding that short-chain fatty acids have an antibacterial effect (Cummings 1981).

However, using enzymatic methods, Mudd *et al.* (1978, 1980) did not find any elevation in bile acid concentration in the faeces of patients with colorectal adenoma. On the other hand, in a companion study the faecal pH of patients with cancer of the colon was found to be significantly higher than that of matched controls (Malhotra 1982, in preparation). Ingenious test systems using salmonella mutants and tissue culture techniques are being investigated (Reddy *et al.* 1980). So far no chemical carcinogen has been isolated nor has any work been done to develop a suitable antibacterial agent that will suppress the offending colonic bacteria. In a recent study, van der Werf *et al.* (1982) have shown that colonic absorption of deoxycholate and duodenal bile was significantly higher in patients with adenomatous polyps than in matched controls. This may accord with both hypotheses put forward for the role of bile in the aetiology of cancer of the colon: a direct contact action of a chemical carcinogen; and a direct mutagenic effect on the mucus of the mucous cells of an increased pH due to higher flow rates of bile, proposed previously (Malhotra 1967*a*; 1976, 1977*a*) and also discussed in this paper. Diet is involved in both mechanisms. As Cummings (1980) has noted, the next few years will probably provide an answer and, should it be found, modification of the diet may well then provide the means for controlling this disease.

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